MICROORGANISMS IN SOLID MATERIALS

TASK II:

NATURALLY OCCURRING MICROBIOLOGICAL FLORA FROM NORMALLY PREPARED PROPELLANT SPECIMENS

(Mod. 1)

VOLUME IV

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SUMMARY

The extent and character of microbiological contamination in each of two batches of solid propellant were measured. In a portion of one batch a population as large as 10^6 cells/milliliter was found. While no thermophiles nor obligate anaerobes were detected, many species of aerobes and facultative anaerobes were. Several of these species appear to be more resistant to ethylene oxide than are spores of B. subtilis, var. niger.

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I. INTRODUCTION

That solid propellants may contain microorganisms more resistant to ethylene oxide than are spores of B. subtilis var. niger was indicated by discovery of species of alpha. Species alpha was found in aerobic incubation of pulverized solid propellant dispersed in Trypticase soy broth and incubated at 37°C. This propellant specimen had been prepared from a formulation which had included both ethylene oxide and an inoculum of B. subtilis var. niger spores. Whether normally prepared solid propellant might also contain anaerobes, thermophiles or molds more resistant than either alpha or B. subtilis var. niger, when embedded in solid propellant, had been unresolved.

Replicated specimens of two types of solid propellant and of a Pfaudler-pot fuel mix (containing Alrosperse, polypropylene glycol, and 1, 1, 1 - trimethylol propane) were assayed for viable cell content and the ethylene oxide resistant species found identified with respect to morphology, Gram-staining characteristics, and colony form and color.

One specimen (SY 251/7) contained aluminum particles while the other (PUP 2/11) did not. Both propellants use a polyurethane-type binder. Alpha was isolated originally from a propellant similar to SY 251/7.

II. METHODS

The solid propellant specimens identified in Table I were assembled in hermetically-sealed, polyethylene bags with the tools and supplies shown in Figure 1. The specimens were in blocks 2-1/2" x 3" x 3" in size. The polyethylene bag was constructed of 4-mil polyethylene film and the seams were sealed by a heat sealing process. In each bag with the propellant specimen and supplies, was placed a jar containing a solution consisting of 100 milliliters of liquid ethylene oxide and 1 milliliter of water. This solution evaporated to saturate the air in the bag with ethylene oxide vapor and maintain the absolute humidity in the bag about that equivalent to 50 percent relative humidity. The concentrations of ethylene oxide and water in the bag were confirmed by gas chromatography. The bags remained sealed for a period of 72 hours before the solid propellant specimens were sampled and pulverized particles dispersed in the previously sterile fluid thioglycollate medium (FTM) in baby food jars.

To sample a solid propellant specimen, it was transferred to one of the two powder collection compartments where it was abraded by the file while dry. All particles were less than one millimeter in greatest dimension with many of them in the micron range of sizes. The specimen and the file were removed from the powder collection compartment and were replaced by a jar containing 100 milliliters of sterile fluid thioglycollate broth. During the surface sterilization process the exterior of the specimen had maximum exposure to ethylene oxide. If ethylene oxide penetrated the specimen, the exterior portion would show maximum effect of this penetration. In Table I the viable particle content of this powder is shown for those specimens which have been identified as "exterior (ETO)".

After approximately one gram of the powder was prepared and the replacement made, the passage between the compartment and the remainder of the bag was then closed using a heat sealer.

The contents of the jar of broth were poured into the hermetically sealed powder collection compartment. After verifying the integrity of the seal, the entire bag and its contents were sonicated for one minute. The broth, now containing the pulverized propellant, was returned to the jar which was then sealed and removed from the plastic envelope. The jar was wiped dry.

Previous abrasion techniques have been performed while the specimen was submerged in broth or water in order to suppress the fire hazard. Under aqueous liquids, the ammonium perchlorate is rapidly extracted from the propellant and the abrasion occludes many organisms while it exposes others. When pulverized in the dry state, the structure of the propellant is preserved during the wetting with broth or water thereby reducing occlusion of viable cells.

On wetting, the powdered propellant readily agglomerates into a clump. On working, this clump can be compacted easily. In testing for viable cells, avoidance of compaction is important and for this reason, the suspension including the clump was sonicated to hasten the penetration of the broth and the solution of the ammonium perchlorate.

After the exterior sample had been removed from the specimen, the specimen was sliced along the dotted lines shown in Figure 1. This cut exposed an interior surface for sampling. The smaller portion of the specimen was then put into the second powder collection compartment and approximately one gram of powder abraded from the freshly cut "interior" surface. The steps for suspending the powder in the broth were repeated.

The gas remaining in the bag with the tools and two blocks of specimen was then assayed for ethylene oxide and water vapor contents. The ethylene oxide content, in every case, was found to be less than 70 milligrams per liter and the water vapor content to exceed that equivalent to 50 percent relative humidity.

The bag for one of the propellant specimens contained, in addition to the tools and supplies discussed above, a microtome, tweezers, and a third jar of broth. After the powder specimens had been collected, a

portion of propellant from the interior of the specimen was placed in the microtome holder and slices approximately 400 microns thick were removed from it and placed in the previously sterile broth. Approximately one gram of propellant was transferred to the broth in this manner.

Using a sterile pipette and aseptic technique, one milliliter of the Pfaudler-pot fuel mix was transferred to the previously sterile broth.

Bacteriostasis controls on the broth were prepared by having a third bottle of broth in two of the bags. These broths were confirmed to be nonbacteriostatic to spores of Cl. sporogenes. After 72 hours incubation, all jars still showing no evidence of bacterial populations were inoculated with one-half milliliter of the fluid from one of the jars showing turbidity. On further incubation, turbidity and a yellow color developed in every one of the jars, verifying absence of strong bacteriostatic effects.

Within two hours after the pulverized specimen was suspended in the previously sterile fluid thioglycollate medium (FTM), a 0.5-milliliter aliquot of the suspension was withdrawn and placed on the surface of Trypticase soy agar (TSA) and incubated there under each of the conditions shown in Table I. In addition, Sabourauds dextrose agar (Sab) and anaerobic agar (AA) in large, deep test tubes were inoculated with 0.5-milliliter aliquots of the suspension and incubated under each of the corresponding conditions shown in Table I.

The jars containing the suspension of pulverized propellant and the Pfaudler-pot fuel mix were incubated at 37°C for 6 hours (10 hours in the case of Specimens No. 4 and 6) after which a second set of plates and AA tubes were inoculated with 0.5-milliliter aliquots. The jars were then returned to the incubator where they remained until a total of 72 hours had elapsed. They were then sampled again for the presence of anaerobes and then tested for bacteriostasis in the manner described above.

All plates were incubated for 7 days before final colony counts were recorded. On the right side of Table I, the information under the heading, "in FTM at 37" describes the appearance of the broth in the baby food jars when they were tested for the presence of anaerobes and bacteriostasis.

Under the heading "on Brewer plates" are recorded the numbers of colonies appearing on the surface of anaerobic agar (AA) inoculated with 0.5 milliliters of the fluid in the incubated baby food jars. Anaerobic incubation was provided by use of Brewer anaerobic plates. In most cases the colonies were mucoid and coalesced to form one large colony.

The integrity of the same closure on these same baby food jars, with respect to ethylene oxide penetration, was established by many tests in earlier laboratory studies.

The footnotes following Table II present specific information about the details of the tests whose results are reported in that table.

III. RESULTS

Table I shows the numbers of naturally occurring microorganisms which can be detected in five milligrams of pulverized solid propellant or Pfaudler-pot fuel mix. Two of the specimens contained in excess of 10^6 viable cells per milliliter. The most prevalent species seemed to be a Gram-negative aerobic bacillus which was not, however, particularly resistant to ethylene oxide in ethylene oxide-screening culture but seemed to survive exposure to surface sterilization. The preparation of the ethylene oxide-screening culture is described in a note following Table II.

On incubation of the suspension of pulverized propellant, the numbers of viable cells of some species seemed to decrease while those of other species seemed to increase. The frequency of occurrence of cells of anaerobic species seemed to parallel the frequency of appearance of cells of aerobic species.

When the plates contain many colonies, the frequency of colonies from two or more viable cells coalescing to appear as one colony increases. In addition, cells which are late in germinating find that the agar has been depleted of nutrients by those cells which developed colonies earlier. Some strains and species produce substances which suppress the proliferation of other strains of microorganisms. In the interpretation of the information in Table I, little reliance can be placed on the numbers recorded for a particular type of colony when either many colonies or many types of colonies were observed. The information in the table does show that never more than five really distinct types of colonies appeared on any one plate and that some plates contained relatively many colonies while other plates contained relatively few. Comparisons of relative numbers of colonies of different species appearing on any particular plate are not meaningful. Differences may be due more to conditions of incubation than anything else.

Earlier work had found that as few as 100 viable spores per milliliter of B. subtilis var. niger in solid propellant, which contained aluminum, were detectable when the propellant was pulverized by shaving with a microtome. No B. subtilis var. niger cells were found in any of the propellant studied in the program reported here, though cells resembling species alpha were found. A variety of reasons why the microtomed specimen failed to show contamination could be offered, but to establish which reasons are correct would have required an effort beyond the scope of the program. It was sufficient to show that the file abrasion of dry propellant was a sufficiently sensitive technique for detection of microbial contamination to be useful.

Table I shows that the microbial contamination was not uniform throughout a single block of cast propellant. This observation may indicate that the microbial contamination was introduced late in the mixing process or during casting. This indication is supported by the failure of the testing technique used to find any viable cells in the Pfaudler-pot fuel mix or any of the ingredients of the propellants. These techniques were not sensitive enough to measure very low orders of contamination but were established to measure moderately large contamination loads.

Table II describes the characteristics of many of the species of microorganisms found in the solid propellant specimens. The characteristics of the species alpha, found earlier in solid propellant, beta, found to be a particularly ethylene oxide-resistant species present in dust at JPL, and phi, identified as a strain of B. lichenformis, have been included in the table. For purposes of colony description the facultative species, other than alpha were grown on anaerobic agar in Brewer anaerobic plates and in fluid thioglycollate medium rather than on the Trypticase soy substrates. The descriptions of colonies on the anaerobic plates may not correspond with those of ethylene-oxide resistance because the anaerobic plates contained mixed cultures of possibly widely varying resistance to ethylene oxide.

By the Greek and Hebrew letters in front of the numbers, Table I shows the source of many of the species of microorganisms described in Table II. The five colony types indicated in Table I do not signify the same types of colonies for all of the plates. The presence of other species and

greater numbers of colonies on some plates than on others causes a change in the sizes and appearances of the colonies. In addition, the appearance of the colonies of a particular species will vary with incubation conditions. For this reason, the set of colonies chosen for identification may contain replications and most likely will.

IV. CONCLUSIONS

1. Several varieties of microorganisms are present in the solid propellant, some to the extent of 10^6 per milliliter. Most of the easily detected species are aerobes though many facultative anaerobes are also present. Thermophiles are few.

2. Among the species of microorganisms found in the solid propellant were several more resistant to ethylene oxide than are spores of B. subtilis var. niger under the conditions of the test.

3. The extent of contamination varies within a single block of cast propellant. The greatest contamination is not necessarily either the first or last portion cast.

4. The microbial contamination in the specimens containing aluminum powder (SY 251/7) produced fewer colonies on the assay plates than did the specimens which contained no aluminum powder. While this effect could have been due to the presence of the aluminum particles, it could equally well be due to the batch-to-batch variation in the preparation of the propellant. The absence of microbial flora in the pot fuel mix indicates that detectable microorganisms were present in numbers fewer than 10 per milliliter on the basis of the sampling statistics. If the pot fuel mix is bacteriostatic to any degree even 10 cells/milliliter may fail to show their viability in the assay procedures used. The difference in numbers of viable microorganisms in the propellant and in the Pfaudler-pot fuel mix indicates that the contamination occurred after this fuel mix was prepared. The work in Task I indicates also that the contamination came either from accidental contamination during the mixing or casting operation or that microorganisms of certain species proliferate in the solid propellant. The specimen which contained the greatest number of viable cells was stored for 8 months before it was sampled and tested for microorganism content.

5. On suspension of microorganisms in broth, the numbers of some species decrease so that the ability to detect the presence of these species depends upon the promptness of the assay of the suspension.

6. Abrasion of the propellant while it is dry produces fine particles from which microorganisms can be recovered at least as well as from microtomed slices 400 microns thick.

7. Under the conditions used, ethylene oxide does not penetrate a block of solid propellant deeply. Some does penetrate deeply enough, however, to reduce the size of the viable population near the surface.

TABLE I

NATURALLY OCCURRING POPULATIONS OF MICROORGANISMS IN NORMALLY PREPARED SOLID PROPELLANT

Numbers of Each of Several Types of Colonies

Specimen	Plate	Colony Type	A 0.5-ml Aliquot of the Suspension[1] of Pulverized Specimen Was Incubated					A 0.5-ml Aliquot of the Suspension of Pulverized Specimen after Six Hours[2] at 37 deg. C was Incubated						
			Aerobically				Anaerobically	Aerobically				Anaerobically		
			on TSA at 55°C	on TSA at 37°C	on TSA at 25°C	on Sab at 25°C	in AA at 37°C	on TSA at 55°C	on TSA at 37°C	on TSA at 25°C	on Sab at 25°C	in AA at 37°C	in FTM at 37°C	on Brewer Plates
1. From interior of a 3"x3" x 2 1/2" block cut from top of a larger block of propellant identified as PUP 2/11	a	1	0	19	29	11	0	0	~ 200	-	~ 500	21	yellow and turbid broth	100
		2	0	~ 1500	~ 400	0	~ 1000	0	~ 1500	-	?	0		
		3	0	0	0	0	0	0	35	-	0	0		
		4	0	~ 100	~ 200	~ 200	0	0	0	-	~ 1000	0		
		5	0	0	0	0	0	0	0	-	0	0		
	b	1	0	28	25	8	0	0	~ 200	-	~ 500	38		
		2	0	~ 1500	~ 400	0	~ 1000	0	~ 1500	-	?	0		
		3	0	0	0	0	0	0	25	-	0	0		
		4	0	~ 100	~ 200	~ 200	0	0	0	-	~ 1000	0		
		5	0	0	0	0	0	0	0	-	0	0		
2. From interior of a 3"x 3" x 2 1/2" block cut from bottom of a larger block of propellant identified as PUP 2/11	a	1	0	19	29	20	0	0	Over-grown by colonies of type number 1	-	~ 2000	0	yellow and turbid broth	Overgrow with confluent colonies
		2	0	~ 1500	~ 500	ρ 80	~1000	0		-	~ 2000	10		
		3	0	0	0	0	0	0		-	0	0		
		4	0	~ 200	~ 150	100	0	0		-	?	0		
		5	0	0	0	0	0	0		-	0	0		
	b	1	0	32	48	30	0	0		-	~ 2000	0		
		2	0	~1500	~ 500	15	~ 1000	0		-	~ 2000	12		
		3	0	0	0	0	0	0		-	0	0		
		4	0	λ ~200	~ 100	~100	0	0		-	?	0		
		5	0	0	0	0	0	0		-	0	0		

[1] One gram (approximately) of the pulverized propellant specimen (or other material) was suspended in 100 ml of fluid thioglycollate medium in a baby food jar.

[2] The pulverized specimen in the fluid thioglycollate medium was stores for six hours at 37°C before a second 0.5 ml of it was put on each of the indicated substrates.

TABLE I (Continued)

Numbers of Each of Several Types of Colonies

Specimen	Plate	Colony Type	A 0.5-ml Aliquot of the Suspension[1] of Pulverized Specimen Was Incubated					A 0.5-ml Aliquot of the Suspension of Pulverized Specimen after Six Hours[2] at 37 deg. C was Incubated						
			Aerobically				Anaerobically	Aerobically				Anaerobically		
			on TSA at 55°C	on TSA at 37°C	on TSA at 25°C	on Sab at 25°C	in AA at 37°C	on TSA at 55°C	on TSA at 37°C	on TSA at 25°C	on Sab at 25°C	in AA at 37°C	in FTM at 37°C	on Brewer Plates
3. From interior of a 3" x 3" x 2 1/2" block cut from top of a larger block of propellant identified as SY 251/7	a	1	0	0	0	0	0	0	0	-	0	0	yellow brown and turbid	overgrown with confluent colonies
		2	0	7	0	3	0	0	1	-	1	0		
		3	0	0	0	0	0	0	0	-	0	0		
		4	0	0	6	0	0	0	0	-	0	0		
		5	0	1	0	0	0	0	0	-	0	0		
	b	1	0	0	0	0	0	0	0	-	0	0		
		2	0	7	0	2	0	0	0	-	0	0		
		3	0	0	0	0	0	0	0	-	0	0		
		4	0	0	6	0	0	0	0	-	0	0		
		5	0	μ 2	2	0	0	0	0	-	0	0		
4. From interior of a 3" x 3" x 2 1/2" block cut from bottom of a block of propellant identified as SY 251/7	a	1	0	θ 2	1	1	0	0	~1000	Over-grown with colonies of type number 1	0	2	yellow and turbid broth	overgrown with confluent colonies
		2	0	ι 160	0	25	~2000	0	~1000		~1000	0		
		3	0	0	0	0	0	0	0		0	0		
		4	0	0	100	25	0	0	0		~1000	0		
		5	0	0	0	0	0	0	0		0	0		
	b	1	0	0	0	1	0	0	~1000	-	0	0		
		2	0	90	0	25	~2000	0	~1000	-	~1000	0		
		3	0	0	0	0	0	0	0	-	0	0		
		4	0	0	70	60	0	0	0	-	~1000	0		
		5	0	0	0	0	0	0	0	-	0	0		

[1]One gram (approximately) of the pulverized propellant specimen (or other material) was suspended in 100 ml of fluid thioglycollate medium in a baby food jar.

[2]The pulverized specimen in the fluid thioglycollate medium was stored for six hours at 37°C before a second 0.5 ml of it was put on each of the indicated substrates. The suspension of the "bottom" specimen was at 37°C for 10 hours before second aliquot was taken.

TABLE I (Continued)

Numbers of Each of Several Types of Colonies

Specimen	Plate	Colony Type	A 0.5-ml Aliquot of the Suspension[1] of Pulverized Specimen Was Incubated					A 0.5-ml Aliquot of the Suspension of Pulverized Specimen after Six Hours[2] at 37 deg. C was Incubated						
			Aerobically				Anaerobically	Aerobically				Anaerobically		
			on TSA at 55°C	on TSA at 37°C	on TSA at 25°C	on Sab at 25°C	in AA at 37°C	on TSA at 55°C	on TSA at 37°C	on TSA at 25°C	on Sab at 25°C	in AA at 37°C	in FTM at 37°C	on Brewer Plates
5. From exterior (ETO) of a 3"x 3" x 2 1/2" block cut from top of a larger block of propellant identified as SY 251/7	a	1	0	0	0	0	0	0	0	–	0	0	brown and clear broth	2000
		2	0	0	0	0	0	0	0	–	0	0		
		3	0	0	0	0	0	0	0	–	0	0		
		4	0	0	0	0	0	0	0	–	0	0		
		5	0	0	0	0	0	0	0	–	0	0		
	b	1	0	0	0	0	0	0	0	–	0	0		
		2	0	0	0	0	0	0	0	–	0	0		
		3	0	0	0	0	0	0	0	–	0	0		
		4	0	0	0	0	0	0	0	–	0	0		
		5	0	1	0	0.	0	0	0	–	0	0		
6. From exterior (ETO) of a 3"x 3" x 2 1/2" block cut from bottom of a larger block of propellant identified as SY 251/7	a	1	0	0	0	0	0	0	0	0	0	0	brown and clear broth	0
		2	0	0	0	0	0	0	0	0	0	0		
		3	0	0	0	0	0	0	0	0	0	0		
		4	0	0	0	0	0	0	0	0	0	0		
		5	0	0	0	0	0	0	0	0	0	0		
	b	1	0	0	0		0	0	0	–	0	0		
		2	0	0	0		0	0	0	–	0	0		
		3	0	0	0		0	0	0	–	0	0		
		4	0	0	0		0	0	0	–	0	0		
		5	0	0	0		0	0	0	–	0	0		

[1] One gram (approximately) of the pulverized propellant specimen (or other material) was suspended in 100 ml of fluid thioglycollate medium in a baby food jar.

[2] The pulverized specimen in the fluid thioglycollate medium was stored for six hours at 37°C before a second 0.5 ml of it was put on each of the indicated substrates. The suspension of the "bottom" specimen was at 37°C for 10 hours before the second aliquot was taken.

TABLE I (Continued)

Numbers of Each of Several Types of Colonies

Specimen	Plate	Colony Type	A 0.5-ml Aliquot of the Suspension[1] of Pulverized Specimen Was Incubated					A 0.5-ml Aliquot of the Suspension of Pulverized Specimen after Six Hours[2] at 37 deg. C was Incubated						
			Aerobically				Anaerobically	Aerobically				Anaerobically		
			on TSA at 55°C	on TSA at 37°C	on TSA at 25°C	on Sab at 25°C	in AA at 37°C	on TSA at 55°C	on TSA at 37°C	on TSA at 25°C	on Sab at 25°C	in AA at 37°C	in FTM at 37°C	on Brewer Plates
2. From exterior (ETO) of a 3" x 3" x 2 1/2" block cut from top of a larger block of propellant identified as PUP 2/11	a	1	0	9	ν 8	π 12	0	0	51	–	45	7	yellow and turbid broth	overgrown with confluent colonies
		2	0	~ 600	ξ ~ 400	3	~ 500	0	~ 500	–	40	0		
		3	0	1	0	0	0	0	7	–	0	0		
		4	0	60	0 ~ 100	τ ~ 100	0	0	~ 50	–	40	0		
		5	0	0	0	0	0	0	0	–		0		
	b	1	0	10	11	5	0	0	22	–	40	8		
		2	0	~ 600	~ 400	2	~ 500	0	~ 500	–	35	0		
		3	0	0	0	0	0	0	κ 4	–	0	0		
		4	0	50	~ 100	~ 100	0	0	~ 50	–	40	0		
		5	0	0	0	0	0	0	0	–	0	0		
3. From exterior (ETO) of a 3"x3"x 2 1/2 block cut from bottom of a larger block of propellant identified as PUP 2/11	a	1	0	1	0	0	0	0	22	–	8	0	yellow and turbid broth	overgrown with confluent colonies
		2	0	2	0	3	30	0	7	–	3	0		
		3	0	0	0	0	0	0	0	–	0	0		
		4	0	0	2	0	0	0	0	–	0	0		
		5	0	0	0	0	0	0	0	–	2	0		
	b	1	0	0	0	0	0	0	20	–	9	0		
		2	0	5	0	1	20	0	3	–	4	0		
		3	0	0	0	0	0	0	0	–	0	0		
		4	0	0	10	0	0	0	0	–	0	0		
		5	0	0	0	0	0	0	0	–	0	0		

[1] One gram (approximately) of the pulverized propellant specimen (or other material) was suspended in 100 ml of fluid thioglycollate medium in a baby food jar.

[2] The pulverized specimen in the fluid thioglycollate medium was stored for six hours at 37°C before a second 0.5 ml of it was put on each of the indicated substrates.

TABLE I (Continued)

Numbers of Each of Several Types of Colonies

Specimen	Plate	Colony Type	A 0.5-ml Aliquot of the Suspension[1] of Pulverized Specimen Was Incubated					A 0.5-ml Aliquot of the Suspension of Pulverized Specimen after Six Hours[2] at 37 deg. C was Incubated						
			Aerobically				Anaerobically	Aerobically				Anaerobically		
			on TSA at 55°C	on TSA at 37°C	on TSA at 25°C	on Sab at 25°C	in AA at 37°C	on TSA at 55°C	on TSA at 37°C	on TSA at 25°C	on Sab at 25°C	in AA at 37°C	in FTM at 37°C	on Brewer Plates
9. From a bottle of Pfaudler-pot fuel mix (a mixture of Alrosperse, PPG, and TMP) identified as SY 239-240-241	a	1	0	0	0	0	0	0	0	–	0	0	yellow brown and turbid	overgrown with confluent colonies
		2	0	0	0	0	0	0	0	–	0	0		
		3	0	0	0	0	0	0	0	–	0	0		
		4	0	0	0	0	0	0	0	–	0	0		
		5	0	0	0	0	0	0	0	–	0	0		
	b	1	0	0	0	0	0	0	0	–	0	0		
		2	0	0	0	0	0	0	0	–	0	0		
		3	0	0	0	0	0	0	0	–	0	0		
		4	0	0	0	0	0	0	0	–	0	0		
		5	0	0	0	0	0	0	0	–	0	0		
10. From a bottle of Pfaudler-pot fuel mix (a mixture of Alrosperse, PPG, and TMP) identified as SY 245-246-247	a	1	0	0	0	0	0	0	0	–	0	0	yellow brown and turbid	overgrown with confluent colonies
		2	0	0	0	0	0	0	0	–	0	0		
		3	0	0	0	0	0	0	0	–	0	0		
		4	0	0	0	0	0	0	0	–	0	0		
		5	0	0	0	0	0	0	0	–	0	0		
	b	1	0	0	0	0	0	0	0	–	0	0		
		2	0	0	0	0	0	0	0	–	0	0		
		3	0	0	0	0	0	0	0	–	0	0		
		4	0	0	0	0	0	0	0	–	0	0		
		5	0	0	0	0	0	0	0	–	0	0		

[1] One gram (approximately) of the pulverized propellant specimen (or other material) was suspended in 100 ml of fluid thioglycollate medium in a baby food jar.

[2] The pulverized specimen in the fluid thioglycollate medium was stored for six hours at 37°C before a second 0.5 ml of it was put on each of the indicated substrates.

TABLE I (Continued)

Numbers of Each of Several Types of Colonies

Specimen	Plate	Colony Type	A 0.5-ml Aliquot of the Suspension[1] of Pulverized Specimen Was Incubated					A 0.5-ml Aliquot of the Suspension of Pulverized Specimen after Six Hours[2] at 37 deg. C was Incubated						
			Aerobically				Anaerobically	Aerobically				Anaerobically		
			on TSA at 55°C	on TSA at 37°C	on TSA at 25°C	on Sab at 25°C	in AA at 37°C	on TSA at 55°C	on TSA at 37°C	on TSA at 25°C	on Sab at 25°C	in AA at 37°C	in FTM at 37°C	on Brewer Plates
1. From interior of a 3"x3" x 2 1/2" block cut from bottom of a larger block of propellant identified as PUP 2/11. Microtome slices were cultured	a	1	0	0	0	0	0	0	0	-	0	0	yellow-brown and turbid	overgrown with confluent colonies
		2	0	0	0	0	0	0	0	-	0	0		
		3	0	0	0	0	0	0	0	-	0	0		
		4	0	0	0	0	0	0	0	-	0	0		
		5	0	0	0	0	0	0	0	-	0	0		
	b	1	0	0	0	0	0	0	0	-	0	0		
		2	0	0	0	0	0	0	0	-	0	0		
		3	0	0	0	0	0	0	0	-	0	0		
		4	0	0	0	0	0	0	0	-	0	0		
		5	0	0	0	0	0	0	0	-	0	0		

[1] One gram (approximately) of the pulverized propellant specimen (or other material) was suspended in 100 ml of fluid thioglycollate medium in a baby food jar.

[2] The pulverized specimen in the fluid thioglycollate medium was stored for six hours at 37°C before a second 0.5 ml of it was put on each of the indicated substrates.

TABLE II

CHARACTERISTICS OF SPECIES OF MICROORGANISMS

RECOVERED FROM SOLID PROPELLANT SPECIMENS

Characteristics	Species Code		
	Alpha	Beta	Theta
CELLS			
Type	coccus	rod	rod
Cell diameter, microns	0.5	1.0	0.9
Cell length, microns	--	5 - 6	3 - 4
Gram-staining characteristics	Gram-positive	Gram-positive granules	Gram-variable terminal granules
Spore-staining characteristics	negative	central spores	central spores
Aggregation	tetrads and diploids	chains	chains
CULTURES			
Oxygen requirements	facultative	aerobe	aerobe
Ethylene oxide resistance[1]	+++	+++	+++
Environmental requirements[2]			
TSA at 55°C	-		-
TSA at 37°C	+++	+++	+++
TSA at 25°C			+
Sab at 25°C			++
AA at 37°C	++		
Populations distribution in broth[3]:			
Uniform	yes	yes	yes
Sediment	yes	yes	yes
Clumps of filaments	in FTM	no	no
Pellicle	no	yes	yes
Location of pellicle	--	top	top
Clear zone at top	in FTM	no	no
Turbidity of culture in broth	medium	dense	medium
COLONIES			
Colony type on agar	smooth	rough	rough
Opacity	translucent	opaque	opaque
Color	gray-white	light yellow	gray-white
Diameter, millimeters	1-3	5	10
Edge	round	lobed	lobed
Texture	shiny	dull	dull
Profile	convex	convex	flat

TABLE II (Continued)

Characteristics	Species Code		
	Iota	Kappa	Lambda
CELLS			
Type	rod	rod	rod
Cell diameter, microns	0.5	0.9	0.6
Cell length, microns	1 - 2	3 - 4	3
Gram-staining Characteristics	negative	Gram-positive	Gram-positive
Spore-staining Characteristics	negative	terminal spores	negative
Aggregation	clusters	single, diploid	clusters
CULTURES			
Oxygen requirements	aerobe	aerobe	aerobe
Ethylene oxide resistance[1]	-	+	+++
Environmental requirements[2]			
TSA at 55°C	-	-	-
TSA at 37°C	+++	+++	+++
TSA at 25°C	++	++	
Sab at 25°C	+	+	
AA at 37°C			
Populations distribution in broth[3]:			
Uniform	--	yes	yes
Sediment	--	yes	yes
Clumps or filaments	--	no	no
Pellicle	--	no	no
Location of pellicle	--	--	--
Clear zone at top	--	no	no
Turbidity of culture in broth	--	light	dense
COLONIES			
Colony type on agar	smooth	smooth	rough
Opacity	transparent	translucent	opaque
Color	light yellow	orange	gray-white
Diameter, millimeters	1/2	3	2
Edge	round	lobed	irregular
Texture	shiny	shiny	dull
Profile	convex	flat	flat

TABLE II (Continued)

Characteristics	Species Code		
	Mu	Nu	Xi
CELLS			
Type	rod	rod	rod
Cell diameter, microns	0.5	0.9	0.5
Cell length, microns	1 - 2	3 - 4	1 - 2
Gram-staining Characteristics	negative	Gram-variable	negative
Spore-staining Characteristics	negative	central spores	negative
Aggregation	single, clusters	chains	clusters
CULTURES			
Oxygen requirements	aerobe	aerobe	aerobe
Ethylene oxide resistance[1]	-	+++	-
Environmental requirements[2]			
TSA at 55°C	-	-	-
TSA at 37°C	++	+++	+++
TSA at 25°C	++	++	++
Sab at 25°C	+	++	+
AA at 37°C			
Population distribution in broth[3]:			
Uniform	--	yes	--
Sediment	--	yes	--
Clumps or filaments	--	no	--
Pellicle	--	yes	--
Location of Pellicle	--	top	--
Clear zone at top	--	no	--
Turbidity of culture in broth	--	dense	--
COLONIES			
Colony type on agar	smooth	rough	smooth
Opacity	translucent	opaque	transparent
Color	yellow	gray-white	light yellow
Diameter, millimeters	2	10	1/2
Edge	round	lobed	round
Texture	shiny	dull	shiny
Profile	convex	flat	convex

TABLE II (Continued)

Characteristics	Species Code		
	Omicron	Pi	Rho
CELLS			
Type	rod	rod	rod
Cell diameter, microns	0.5	0.9	0.5
Cell length, microns	1 - 2	3 - 4	1 - 2
Gram-staining Characteristics	negative	Gram-positive Terminal Granules	negative
Spore-staining Characteristics	negative	central spores	negative
Aggregation	clusters	chains	clusters
CULTURES			
Oxygen requirements	aerobe	aerobe	aerobe
Ethylene oxide resistance[1]	-	-	-
Environmental requirements[2]			
TSA at 55°C	-	-	-
TSA at 37°C	+++	+++	+++
TSA at 25°C	++	++	++
Sab at 25°C	+	+	+
AA at 37°C			
Populations distribution in broth[3]:			
Uniform	--	--	--
Sediment	--	--	--
Clumps or filaments	--	--	--
Pellicle	--	--	--
Location of pellicle	--	--	--
Clear zone at top	--	--	--
Turbidity of culture in broth	--	--	--
COLONIES			
Colony type on agar	smooth	rough	smooth
Opacity	transparent	opaque	transparent
Color	light yellow	gray-white	light yellow
Diameter, millimeters	1	7	1/2
Edge	round	lobed	round
Texture	shiny	dull	shiny
Profile	convex	flat	convex

TABLE II (Continued)

Characteristics	Species Code		
	Sigma	Tau[5]	Upsilon[5]
CELLS			
Type	rod	coccus	coccus
Cell diameter, microns	0.5	0.6 - 0.8	0.8 - 1.0
Cell length, microns	1 - 2	--	--
Gram-staining Characteristics	negative	Gram-positive	Gram-positive
Spore-staining Characteristics	negative	--	--
Aggregation	clusters	diploid, tetrad, small irregular clusters	small irregular clusters
CULTURES			
Oxygen requirements	aerobe	facultative	facultative
Ethylene oxide resistance[1]	-	+	+++
Environmental requirements[2]			
TSA at 55°C	-		
TSA at 37°C	+++		
TSA at 25°C	++		
Sab at 25°C	+		
AA at 37°C	-	+	+
Populations distribution in broth[3]:			
Uniform	--	no	no
Sediment	--	none	none
Clumps or filaments	--	filaments	no
Pellicle	--	--	yes
Location of pellicle	--	--	upper zone
Clear zone at top	--	yes	no
Turbidity of culture in broth	--	filaments	confined to upper zone
COLONIES			
Colony type of agar	smooth	smooth	smooth
Opacity	translucent	opaque	opaque
Color	yellow	white	white
Diameter, millimeters	1	1 - 2	1 - 2
Edge	round	round	round
Texture	shiny	shiny	shiny
Profile	convex	convex	convex

TABLE II (Continued)

Characteristics	Species Code		
	Phi[4]	Chi[5]	Psi[5]
CELLS			
Type	rod	rod	rod
Cell diameter, microns	0.5 - 0.9	0.9	1 - 1.5
Cell length, microns	3 - 4	4 - 5	4.0
Gram-staining Characteristics	Gram-positive terminal granule	Gram-positive	Gram-positive
Spore-staining Characteristics	terminal spores	negative	negative
Aggregation	singles and chains	chains	chains
CULTURES			
Oxygen requirements	aerobe	facultative	facultative
Ethylene oxide resistance[1]	+	+++	+++
Environmental requirements[2]			
TSA at 55°C			
TSA at 37°C	+++		
TSA at 25°C			
Sab at 25°C			
AA at 37°C		+	+
Populations distribution in broth[3]:			
Uniform	yes	no	no
Sediment	no	none	none
Clumps or filaments	no	no	no
Pellicle	yes	yes	yes
Location of pellicle	top	upper zone	upper zone
Clear zone at top	no	no	no
Turbidity of culture in broth	dense	confined to upper zone	confined to upper zone
COLONIES			
Colony type on agar	rough	mucoid	mucoid
Opacity	opaque	translucent	translucent
Color	light yellow	cream	cream
Diameter, millimeters	6 - 10	2 - 3	2 - 3
Edge	lobed-rhizoid	irregular	irregular
Texture	dull	membranous	membranous
Profile	flat	flat	flat

TABLE II (Continued)

Characteristics	Species Code		
	Omega[5]	Aleph[5]	Beth[5]
CELLS			
Type	coccus	coccus	coccus
Cell diameter, microns	0.8 - 1.0	0.6 - 0.8	0.8 - 1.0
Cell length, microns	--	--	--
Gram-staining Characteristics	Gram-positive	Gram-positive	Gram-positive
Spore-staining Characteristics	--	--	negative
Aggregation	diploid, tetrad, small clusters	single, diploid, triad and tetrad	single, diploid, triad, tetrad, small clusters
CULTURES			
Oxygen requirements	facultative	facultative	facultative
Ethylene oxide resistance[1]			++
Environmental requirements[2]			
TSA at 55°C			
TSA at 37°C			
TSA at 25°C			
Sab at 25°C			
AA at 37°C	+	+	+
Populations distribution in broth[3]:			
Uniform	--	--	no
Sediment	--	--	none
Clumps or filaments	--	--	no
Pellicle	--	--	yes
Location of pellicle	--	--	upper zone
Clear zone at top	--	--	no
Turbidity of culture in broth	--	--	confined to upper zone
COLONIES			
Colony type on agar	smooth	smooth	smooth
Opacity	opaque	opaque	opaque
Color	white	white	white
Diameter, millimeters	1 - 2	1 - 2	1 - 2
Edge	round	round	round
Texture	shiny	shiny	shiny
Profile	convex	convex	convex

TABLE II (Continued)

Characteristics	Species Code		
	Gimel[5]	Daleth[5]	He[5]
CELLS			
Type	rod	coccus	rod
Cell diameter, microns	0.9	1.0 - 1.5	0.9
Cell length, microns	4.0	--	4.0
Gram-staining Characteristics	Gram-positive	Gram-positive	Gram-positive
Spore-staining Characteristics	Central spore	--	negative
Aggregation	Single and chain	tetrad, small clusters	single, chain
CULTURES			
Oxygen requirements	facultative	facultative	facultative
Ethylene oxide resistance[1]	+++	+	++
Environmental requirements[2]			
TSA at 55°C			
TSA at 37°C			
TSA at 25°C			
Sab at 25°C			
AA at 37°C	+	+	+
Populations distribution in broth[3]:			
Uniform	no	no	no
Sediment	none	none	none
Clumps or filaments	no	none	no
Pellicle	yes	yes	yes
Location of pellicle	upper zone	upper zone	upper zone
Clear zone at top	no	no	no
Turbidity of culture in broth	confined to upper zone	confined to upper zone	confined to upper zone
COLONIES			
Colony type on agar	mucoid	smooth	mucoid
Opacity	translucent	opaque	translucent
Color	cream	white	cream
Diameter, millimeters	2 - 3	1 - 2	2 - 3
Edge	irregular	round	irregular
Texture	membranous	shiny	membranous
Profile	flat	convex	flat

TABLE II (Continued)

Characteristics	Species Code		
	Cl. sporogenes[5]	Zayin[5]	Cheth
CELLS			
Type	rod	rod	mold spore
Cell diameter, microns	0.5	0.9	3 - 4
Cell length, microns	3.0	4.0	5 - 7
Gram-staining Characteristics	Gramm-positive	Gram-positive	
Spore-staining Characteristics	Terminal spore		
Aggregation	single, chain	single, chain	
CULTURES			
Oxygen requirements	anaerobe	facultative	aerobe
Ethylene oxide resistance[1]	+	+++	
Environmental requirements[2]			
TSA at 55°C			
TSA at 37°C			
TSA at 25°C			
Sab at 25°C			++
AA at 37°C	+	++	
Populations distribution in broth[3]:			
Uniform	no	no	
Sediment	none	none	
Clumps or filaments	no	no	
Pellicle	yes	yes	
Location of pellicle	middle third	at the top	
Clear zone at top	yes	yes	
Turbidity of culture in broth		dense	
COLONIES			
Colony type on agar	mucoid	smooth	
Opacity	translucent	transparent	opaque
Color	cream	light yellow	green
Diameter, millimeters	2 - 3	1	20
Edge	irregular	round	round
Texture	membranous	shiny	felt like
Profile	flat	convex	flat, concentric rings

TABLE II (Continued)

Footnotes

1. Ethylene oxide resistance was measured by a screening test which determined whether or not the species could grow in fluid culture in the presence of ethylene oxide in aqueous solution. The ethylene oxide-screening broth was prepared by mixing into sterile broth a solution of one milliliter of cold liquid ethylene oxide in nine milliliters of cold water in the ratio 1 of solution to 25 of broth. Trypticase soy broth was used for all species except those identified by a superscript "5". For these, fluid thioglycollate medium was used. This screening test was based on a procedure developed in Task I which permitted comparison with the results obtained by other investigators.

   The ethylene oxide resistance of species omega and alpha were not measured because only one colony on each of their respective initial identifying agar plates was sufficiently isolated that a mixed population could be avoided. The minus sign for ethylene-oxide resistance indicates that this species would not grow in the ethylene oxide-screening broth whereas the plus signs indicate that it did grow. The greater the number of plus signs the greater the vigor of the observed growth in the screening broth. One or more colonies identified in Table I were collected from the surface of the agar and transferred on a loop to the broth for incubation. No attempt was made to quantitate the infectivity of the species for ethylene oxide-screening broth.

2. The environmental requirements tests for aerobes were made by streaking the surface of the indicated agars with a loop of the inoculum taken from a colony identified in Table I. The population appeared as a continuous colony along the streak. A single plus sign indicates a visible colony, two plus signs indicate a moderately dense population, and three plus signs indicate very vigorous growth along

the streak. A blank indicates that no test was made.

The Trypticase soy agar (TSA) and the Sabourauds dextrose agar (Sab) were obtained from Hyland Laboratories as Pre-Med Monoplates. The anaerobic agar (AA), fluid thioglycollate medium (FTM), and Trypticase soy broth (TSB) were prepared from dry powder supplied by Baltimore Biological Laboratory.

The environmental requirements tests for the facultative species were made by observing colonies on the surface of anaerobic agar and in deep tubes of anaerobic agar.

3. The observations about the cultures of the several species were made on tubes containing the species in the ethylene-oxide screening cultures described in footnote No. 1 above. Some of the species were grown in fluid culture without ethylene oxide. The presence of ethylene-oxide did not change the appearance of the culture. In most cases, however, the cultures in FTM appeared quite different from cultures of the same species in TSB. The aerobes confined their growth to a band at the top of the broth but large amounts of cells collected at the interfaces between the aerobic and the anaerobic zones to form a structure resembling a pellicle. Below this interface, the cells formed a clumpy turbidity with varying amounts of clear broth. In TSB, the aerobes produced a more or less uniform turbidity. In every case population growth in FTM removed all shades of red color from the broth.

4. This species did not occur in the solid propellant but has occurred as a contaminate in our laboratory. It was present in large numbers in the dust specimens studied in an earlier program. It grows very rapidly and suppresses the growth of alpha when the two occur together. It has been identified as B. lichenformis sp.

5. The colonies of these organisms were isolated initially by incubation of an inoculum taken from the incubated baby food jars and placed on the surface of anaerobic agar in a Brewer anaerobic dish. They were incubated at 37°C.

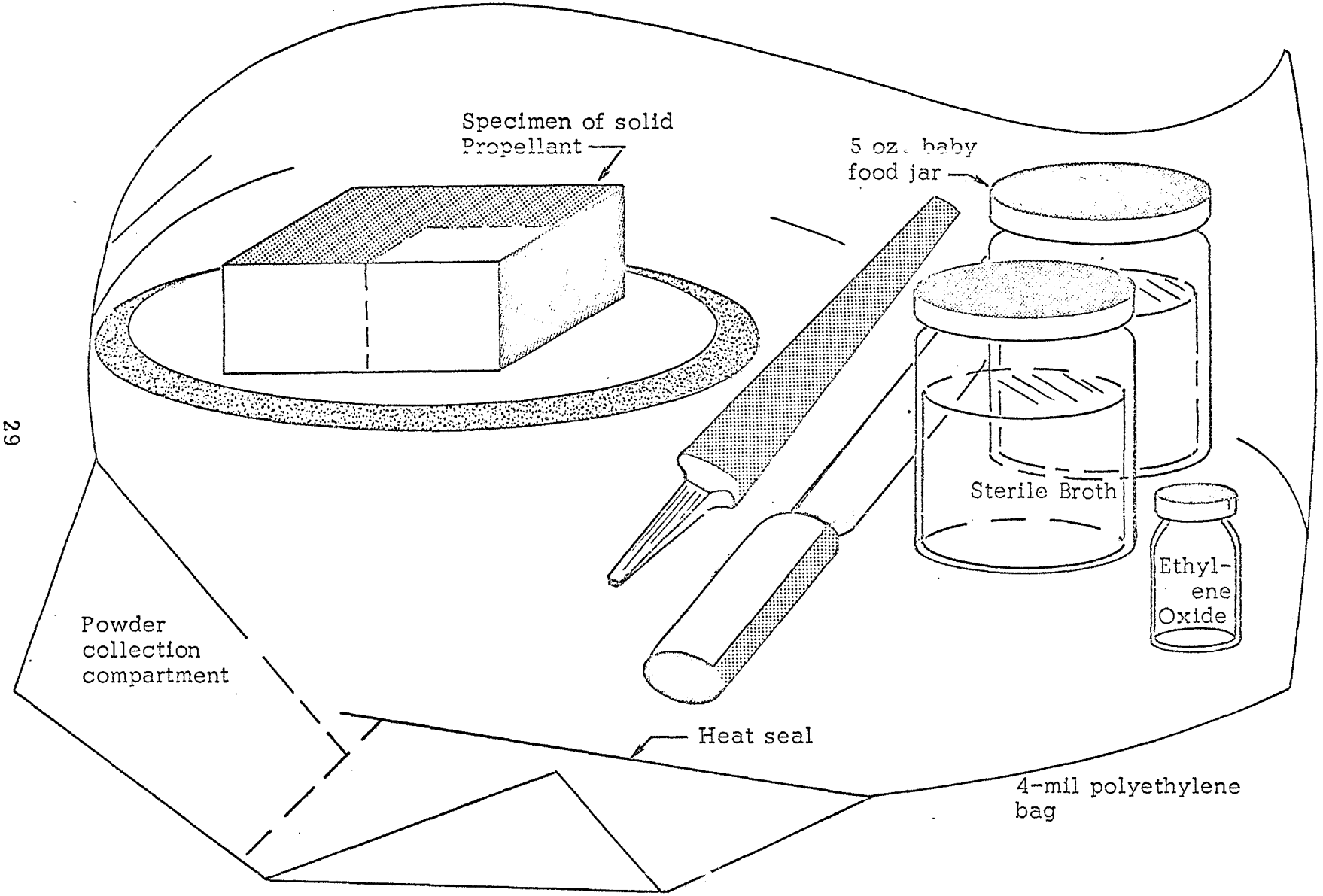

Figure 1. Isolator, Specimen, Tools, and Supplies for Sampling Solid Propellant Specimen

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